Esmethadone (REL-1017) Reduces NMDA Receptor Currents in a Concentration Dependent Manner

Ezio Bettini,1 Corrado Carignani,1 Sara DeMartin,2 Charles E Inturrisi,2 Andrea Matterei,1 Marco Pappagallo,1,3 Steven M Stahl,1 Sergio Travassos,1 Paolo L Manfredi1
1Abipot an Evotec Company, Verona, Italy; 2University of Padova, Italy; 3Relmada Therapeutics, New York, NY, USA; Albert Einstein College of Medicine, Bronx, NY, USA; 4University of California, San Diego, CA, USA; 5Neuroscience Education Institute, San Diego, CA, USA

INTRODUCTION

• Esmethadone (REL-1017; dextromethadone; DXT) is a novel NMDA receptor (NMDAR) antagonist currently in Phase 3 trials for the treatment of major depressive disorder (MDD).

OBJECTIVES

• To examine esmethadone role as an NMDA channel blocker in continuous presence of low L-glutamate concentration, as it might occur in pathological conditions, we characterized esmethadone ability to reduce NMDAR mediated current, after a 120 s perfusion period in the presence of 1 µM L-glutamate and extracellular 1 mM magnesium, at -60 mV membrane potential.

METHODS

• CHO cells stably expressing recombinant diterimeric human NMDARs, co-expressing hGluN1 with hGluN2A, hGluN2B, hGluN2C, or hGluN2D subunits, were used in manual whole cell patch clamp experiments.

• Cells were clamped at -60 mV holding potential, in the presence of 1 mM extracellular MgCl2.

• Voltage protocol included depolarizing 2 seconds step pulses to +40 mV, followed by 2 second ramps back to holding potential.

• The voltage stimulation was repeated 5 times at 15 s intervals.

• Intracellular solution was composed of (in mM): 80 CsCl, 50 CsAc, 0.5 CaCl2, 10 HEPES, 11 EGTA, adjusted to pH 7.25 with CsOH.

• Extracellular solution was composed of (in mM): 150 NaCl, 3 KCl, 1.0 MgCl2, 1.5 CaCl2, 10 HEPES, 10 D-glucose; pH 7.4 with NaOH.

• NMDAR mediated currents were measured -60 mV after 120 s perfusion with 1 µM L-glutamate, first in the absence, then in the presence of 1, 3, 10, 30, or 100 µM esmethadone.

• Current values in presence of esmethadone were percentualized to current values previously recorded in absence of esmethadone and expressed as mean ± standard error mean (SEM).

• Concentration response curve data were fitted by GraphPad Prism to four parameters logistic equation:

\[ I(\%) = 100 / (1 + 10^{(logIC_{50} - log(esmethadone)) \times \text{Hill slope}}) \]

CONCLUSIONS

• Esmethadone reduced 1 µM L-glutamate-induced current in all NMDAR isoforms, in the presence of 1 mM extracellular magnesium and 10 µM glycine.

• Esmethadone showed preference for NMDAR containing GluN2D subunit.

• Esmethadone preference for GluN2D subunit, in the presence of relatively low glutamate concentration, may play a role in its therapeutic antidepressant effect and may help improve our understanding of the pathophysiology of MDD.

DISCLOSURES

• This research was sponsored by Relmada Therapeutics, Inc. Drs. Inturrisi, Stahl, Pappagallo, and Manfredi are paid consultants for Relmada Therapeutics. Drs. Inturrisi and Manfredi are inventors on esmethadone patents and other patents and patent applications.

Figure 1

The scheme application and voltage protocol diagram. Cells were kept at -60 mV, stepped to +40 mV for 2 s, then ramped back to -60 mV (20 s). The voltage stimulation was repeated five times, with 15 s interval. Meanwhile, a 120 s perfusion of 1 µM L-glutamate was performed, in presence of 1 mM glycine and 1 mM MgCl2, and the current value measured as the average of last 2 s (dotted box).

Figure 2

The effect of different esmethadone concentrations was tested in presence of 1 µM L-glutamate for 120 s. Here, sample traces of recordings from cells before and after addition of 30 µM esmethadone are shown. Normalized data were used for the graphs reported in figure 3 and then to evaluate esmethadone IC50 for the various NMDARs. 1 µM L-glutamate was added for 120 s in presence of 10 µM glycine and 1 mM MgCl2. Analysed data are presented in figure 3 and Table 1.

Figure 3

Esmethadone resulted more potent in blocking NMDAR containing hGluN2D subunit (green dots and viscous) in described assay conditions. Graph represents % current recorded in the presence of 1, 3, 10, 30, or 100 µM esmethadone and normalized with respect to control and relative fits are in four different NMDAR cell lines. Recordings were obtained in presence of 1 µM L-glutamate 10 mM glycine and 1 mM MgCl2, at the end of a 120 s incubation period with L-glutamate and esmethadone. Data are mean ± 50SEM. IC50 and Hill slope values of every fitting are reported in Table 1.

Table 1

Esmethadone resulted more potent in blocking NMDAR containing hGluN2D subunit, about 5-fold more potent than when NMDAR contained hGluN1A subunit. Fitting parameters for esmethadone were obtained from data shown in figure 3, and analyzed with GraphPad Prism.

<table>
<thead>
<tr>
<th>Subunits</th>
<th>IC50 (µM)</th>
<th>Hill slope</th>
<th>Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGluN1-hGluN2A</td>
<td>63.1</td>
<td>1.06</td>
<td>2-8</td>
</tr>
<tr>
<td>hGluN1-hGluN2B</td>
<td>41.7</td>
<td>1.17</td>
<td>2-7</td>
</tr>
<tr>
<td>hGluN1-hGluN2C</td>
<td>28.4</td>
<td>1.49</td>
<td>2-8</td>
</tr>
<tr>
<td>hGluN1-hGluN2D</td>
<td>13.5</td>
<td>1.42</td>
<td>3-7</td>
</tr>
</tbody>
</table>